

Genetic markers associated with resistance to infectious hematopoietic necrosis in rainbow and steelhead trout (*Oncorhynchus mykiss*) backcrosses

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Abstract

Backcrosses of rainbow trout and steelhead (*Oncorhynchus mykiss*) were used to construct a linkage map and to identify associations between molecular markers and quantitative trait loci (QTL) determining resistance to infectious hematopoietic necrosis virus (IHNV). The sire map covers 1019 centimorgans (cM), and it is composed of 257 molecular markers [185 amplified fragment length polymorphisms (AFLPs) and 72 microsatellites] distributed over 28 linkage groups. The dam map consists of 236 markers (164 AFLPs and 72 microsatellites), with a total of 45 linkage groups, covering 2041 cM. Sixty-one new microsatellite markers were added to the existing rainbow trout linkage maps. Six AFLPs and six microsatellites in the dam map were associated with IHNV resistance and were located in linkage groups 3, 14, 16, 30, 31, and 39. Sixteen AFLPs and six microsatellites associated with IHNV resistance were located in linkage groups 11, 20, and 25 in the sire map. Microsatellite markers mapped to the three linkage groups were used to screen nine other families, and QTL-marker associations were observed for four of the markers in at least one of the families. Further marker mapping is

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required to increase resolution and to ultimately facilitate positional cloning of QTLs affecting IHNV resistance.

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1. Introduction

Infectious hematopoietic necrosis (IHN) is a highly infectious disease that affects important species of salmon and trout (salmonids) that are cultured both commercially and as part of federal and state sport fisheries enhancement programs. Under the intensive conditions of aquaculture, the IHN virus (IHNV) can cause significant losses at nearly all stages of production. Its overall economic impact in the U.S. rainbow trout industry is estimated to be in the millions of dollars annually (LaPatra et al., 2001). Currently, there is no proven, cost-effective method for IHNV prevention or treatment. Therefore, there is great economic interest in developing a rainbow trout lineage that possesses improved resistance for this pathogen. To accomplish this goal, it will be useful to identify genes that affect IHN resistance. The use of genetic markers to conduct appropriately designed linkage studies is a strategy for finding these important genes. Genetic maps can be used to develop experiments to detect linkage between DNA markers and quantitative trait loci (QTL). There are currently three published rainbow trout maps (Young et al., 1998; Sakamoto et al., 2000; Nichols et al., 2003). Marker-assisted selection (MAS) can then be applied by use of markers linked to QTLs of interest (Lander and Botstein, 1989; Lande and Thompson, 1990).

LaPatra (unpublished data) showed that certain strains of steelhead and steelhead×rainbow trout (*Oncorhynchus mykiss*) crosses are significantly less susceptible to IHNV than commercial strains of rainbow trout. Thus, viral resistance can potentially be transferred to rainbow trout through backcrosses. Furthermore, early survival and growth performance of steelhead×rainbow trout crosses were comparable to that of the pure strain rainbow trout, in the absence of IHNV. The considerable variation in IHNV susceptibility among families of steelhead and rainbow trout should enable identification of DNA markers that cosegregate with this trait. Additionally, rainbow trout form large full-sibling families, which facilitates the QTL mapping process.

In this study, we identified associations of molecular markers with IHNV resistance, and observed some of the associations repeated in different families. We also generated sire and dam linkage maps, composed of amplified fragment length polymorphisms (AFLPs) and microsatellite markers.

2. Materials and methods

2.1. Reference families

Crosses between steelhead (relatively resistant) and rainbow trout (relatively susceptible) formed several F_1 families (Fig. 1). Backcrosses of members of each F_1

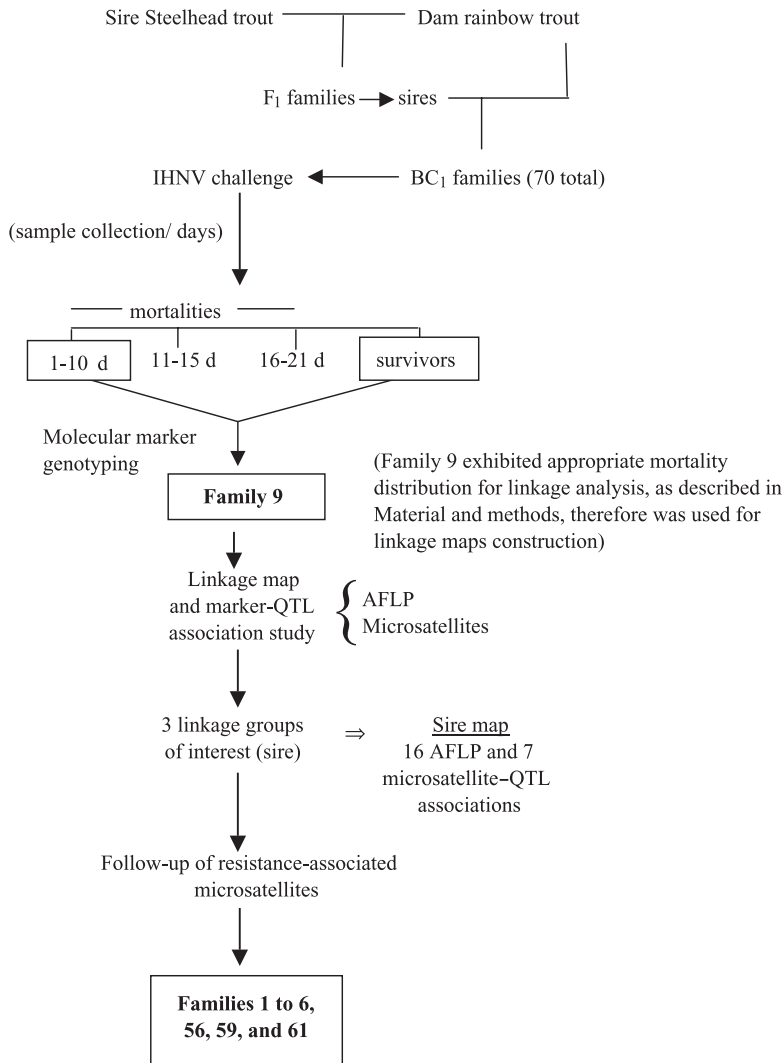


Fig. 1. Experimental design.

family with rainbow trout produced the BC₁ generation. Male steelhead trout (Oxbow strain) were obtained from the Idaho Department of Fish and Game. Rainbow trout used in the crosses were obtained from Clear Springs Foods, Buhl, ID.

2.2. Challenge and sample collection

Two groups of approximately 100 individuals (58 days old) from each family from both generations (F₁ and BC₁) were exposed for 1 h to IHN virus [10,000 plaque-

forming units (PFU)/mL] in a volume of water $10\times$ the total weight of the fish. The virus used in this study was a 1990 isolate of IHNV (220-90; LaPatra et al., 1994) obtained from the Hagerman Valley, ID. Individuals that died during the 21-day monitoring period were collected and stored in 95% ethanol in three different containers: mortalities from day 1 through day 10 of the challenge; days 11 through 15; and days 16 through 21. Challenge survivors and parental fin clips were also collected and stored in 95% ethanol.

2.3. DNA sample extraction and quantification

DNA from survivors and challenge mortalities from families 1 through 6, 9, 56, 59, and 62 were extracted for segregation analysis. Family 9 was chosen to begin the development of the linkage map based on the binomial distribution of survivorship during the IHNV challenge; that is, several individuals died at the beginning of the viral challenge and several survived, suggesting that a gene for IHNV resistance was segregating in this family.

2.3.1. Family 9

DNA from fin clips from 22 individuals that died between days 1 and 10 of the challenge, from 22 survivors, and from their parents, was extracted with a standard phenol/chloroform protocol (Saghai-Marooof et al., 1984; Doyle and Doyle, 1987) as modified by Grewe et al. (1993). DNA was quantified with a Hoeffer TKO 100 fluorometer. Later in the study, we extracted DNA from 20 more individuals from each group (Qiagen DNA extraction kits) that were used in the analysis of a limited number of markers that appeared to be associated with IHNV resistance, as specified in our results.

2.3.2. Families 1 through 6

These BC₁ families were also chosen according to the mode and frequency of mortality of individuals during the challenge process. They are unrelated to family 9; however, some are related to one another (Fig. 2). Qiagen DNA extraction kits were used to extract the DNA from fin clips of 20 offspring from each group (mortalities and survivors) and their parents.

2.3.3. Families 56, 59, and 61

Sigma DNA extraction kits were used to isolate the DNA from 22 challenge mortalities, 22 survivors, and parents from each of these families. A Molecular Dynamics 595 Fluorimager imaging system was used to quantify the DNA samples from families 1 through 6, 56, 59, and 61.

2.4. AFLP

AFLP analyses were conducted according to the protocol of Vos et al. (1995), with some modifications in the PCR cycle. Genomic DNA was digested with two restriction endonucleases, *EcoRI* and *MseI*. *EcoRI* and *MseI* double-stranded

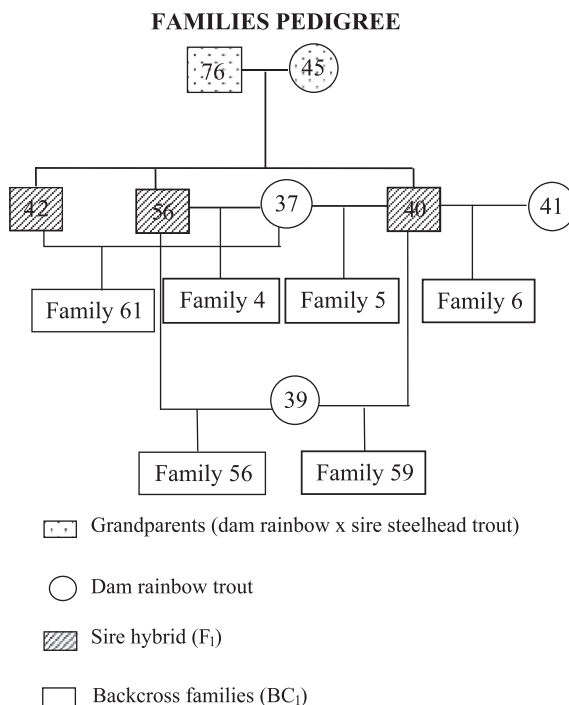


Fig. 2. Pedigree of related families.

adaptors were ligated to the sticky ends of the DNA fragments. In the preselective amplification step, two primers that were complementary to the adaptor/restriction site sequences plus one nucleotide extension at the 3' end were used for selectivity: *MseI*+C and *EcoRI*+G. In the selective amplification step, fluorochrome-labeled primers consisting of sequences that were complementary to the adaptor/restriction sites plus three nucleotides at the 3' end were used for selectivity: *EcoRI*-GNA and GNT; and *MseI*-CNN (where N stands for any of the four possible nucleotides). Every possible combination was examined, for a total of 124 *EcoRI/MseI* primer pairs.

2.5. AFLP reactions

Approximately 2.36 μL of digestion mix (2 μL 10 \times NEBuffer 2, 0.16 μL BSA 1 mg/ μL , 0.1 μL *MseI* enzyme 4 U/ μL , and 0.1 *EcoRI* enzyme 20 U/ μL) to 4 μL of genomic DNA (50 ng/ μL) was added, and digested for 1 h at 37 $^\circ\text{C}$. About 4 μL of the digestion mix/genomic DNA was transferred to the ligation mix (0.4 μL *MseI* adaptor 50 μM , 0.4 *EcoRI* adaptor 5 μM , 0.2 μL ATP 100 mM, 0.2 μL T4 DNA ligase Gibco, 0.8 μL T4 DNA ligase buffer 10 \times Gibco, 0.1 BSA 1 mg/mL, 1.9 dd H_2O) and incubated at 25 $^\circ\text{C}$ for 12–16 h. The solution was then diluted with 80 μL of TE (10 mM Tris; 0.1 mM EDTA) and stored at $-20\text{ }^\circ\text{C}$.

2.5.1. Preselective amplification

We used 20 μL of PCR reaction containing 8.72 μL dd H_2O , 2 μL 10 \times PCR Buffer, 1.6 μL dNTP (2.5 mM each), 0.6 μL MgCl_2 (50 mM), 0.08 μL *Taq* polymerase (5 U/ μL), 1 μL *Eco* G primer (6 μM), 1 μL *Mse* C primer (6 μM), and 5 μL template DNA (diluted digestion–ligation mix). Reactions were amplified in an MJ PTC-100-96 V thermocycler, with the following parameters: 94 °C for 90 s followed by 23 cycles at 94 °C for 30 s, 56 °C for 60 s, and 72 °C for 60 s. Preamplification products were diluted with 180 μL TE and stored at –20 °C.

2.5.2. Selective amplification

The same reagents were used as in the preselective amplification step, but the primers and the DNA template. We added 1 μL *Mse*I CNN primer (5 μM), 1 μL *Eco*RI GNA or GNT primers (1 μM), and 5 μL PCR product from the preselective step as DNA template; PCR products were stored at –20 °C. The template was amplified with the following PCR cycle: 12 cycles at 94 °C, for 30 s, 65 °C for 30 s, and 72 °C for 60 s, with an annealing temperature reduction of 0.7 °C for each cycle, followed by 21 cycles with annealing temperature at 56 °C for 30 s.

2.6. Molecular markers—microsatellites

The microsatellite primers used in this study were obtained from various sources: *Ots*G (University of California Davis, B. May; some loci are published in Williamson et al., 2002), *Ogo* (Olsen et al., 1998), *One* μ (Scribner et al., 1996), *Omy*77 (Morris et al., 1996), *FGT* (Sakamoto et al., 1994a,b; Tokyo University of Fisheries, Japan, N. Okamoto—personal communication), *Ots*100 and *Ots*107 (Nelson and Beacham, 1999), *Ots*101 (Small et al., 1998), *Ssa*289 (McConnell et al., 1995), *Ssa*403UOS (Cairney et al., 2000), *Ssa*-NVH (Norwegian College of Veterinary Medicine, Norway, B. Hoyheim—personal communication), *OMM*1000 to 1050 (Rexroad et al., 2002a), *OMM*1053–1125 (Rexroad et al., 2002b), and *Minisat* (Cordes et al., 2001).

For amplifications of individual samples, the PCR mix consisted of 4.95 μL dd H_2O , 1 μL 10 \times PCR Buffer, 0.8 μL dNTP (2.5 mM each), 0.4 μL MgCl_2 (50 mM), 0.05 μL *Taq* polymerase (5 U/ μL), 0.8 μL primer 1 (10 $\mu\text{M}/\mu\text{L}$), 0.8 μL primer 2 (10 $\mu\text{M}/\mu\text{L}$), and 2 μL DNA template (5 ng/ μL).

Families 1 to 6 were prescreened for 13 microsatellites (Table 1). For each family, equal amounts of DNA were pooled from 10 mortalities and 10 survivors (two of each), and screened for band intensity differences (Fig. 3). Markers showing band intensity differences between the group pools were used to genotype 20 survivors and 20 mortalities to test for cosegregation with the trait (Fig. 4). For the pooled samples, DNA templates were diluted to 20 ng/ μL (dd H_2O). The diluted templates from 10 individuals were pooled together to yield a final concentration of 2 ng/ μL /individual sample or 20 ng/ μL /pooled sample). The PCR master mix for pooled samples contained 6.6 μL dd H_2O , 2 μL 10 \times PCR buffer, 1.6 μL dNTP (2.5 mM each), 0.6 μL MgCl_2 (50 mM), 0.2 μL *Taq* polymerase (5 U/ μL), 2 μL primer 1 (10 $\mu\text{M}/\mu\text{L}$), 2 μL primer 2 (10 $\mu\text{M}/\mu\text{L}$), and 5 μL DNA template (20 ng/ μL). The following PCR cycle was used: 95 °C for 90 s, and 29 cycles at 95 °C for 60 s, 52 °C for 30 s, and 72 °C for 60 s.

Table 1
Results of bulked segregation analyses

Linkage group (fam. 9)	Microsatellites	Families								
		1	2	3	4	5	6	56	59	61
11	<i>Ssa7NVH</i>	P	X	P	P	P	P			
	<i>OtsG78</i>	P	P	P	P	P	P			
	<i>OMM1045</i>	P	P	P	P	P	P			
	<i>OMM1105</i>	P	P	P	X	P	P			
20	<i>FGT1</i>	P	X	P	P	X	P			
	<i>Ssa94NVH</i>	P	X	X	X	X*	X	X	X	X
	<i>OtsG3</i>	X	X	X	X	X	X			
	<i>Ssa10NVH</i>	P	P	P	P	P	P			
25	<i>OMM1021</i>	P	P	P	X	P	P			
	<i>Oneμ8^b</i>	P	X	P	P	P	X*		X	
	<i>OMM1054</i>	X	X*	P	X	X	X*	X	X	X
	<i>Ogo2</i>	P	X	P	P	P	X*		X	
	<i>Ssa121NVH</i>	P	P	X	P	P	P			

Families 1 to 6 were pooled and amplified for 13 microsatellites located in linkage groups 11, 20, and 25 of family 9 sire's map (marked as P). The markers showing band intensity difference between the mortality and survivor groups (marked as X) were amplified individually for observation of band cosegregation with trait.

* Loci associated with IHN resistance through Chi-square test ($p < 0.05$).

2.7. Electrophoresis

Equal amounts of loading buffer (98% formamide, 10 mM EDTA) were added to the amplification products. The solution was denatured (95 °C for 4 min) and placed on ice. Samples were loaded on a 5% polyacrylamide/7.5% urea denaturing gel, which was prewarmed to 50 °C. AFLP products were electrophoresed at 35 W for 60–90 min, and microsatellites were electrophoresed at 30 W for 60 min.

2.8. Scoring and band analysis

The microsatellite gels were stained with Syber Green (50 ml dd H₂O, 1 g agarose, 0.75 μL Syber Green) for 10 min, following the protocol of Rodzen et al. (1998). AFLP fragments amplified with fluorescently labeled primers were visualized with a Molecular Dynamics Fluorimager 595 imaging system. The size of the segregating bands was determined with the software FragneNT Analysis.

2.9. Segregation analysis

Amplified AFLP and microsatellite fragments were scored as “present” or “absent”. Only the informative bands were scored, i.e., those for which one of the parents was homozygous absent and the other parent heterozygous. In the case of the AFLP markers, we determined that a parent was informative for a marker when it presented a band that its mate did not share, and presence and absence of the band was observed among the offspring. Because the challenged offspring were the result of a cross between outbred

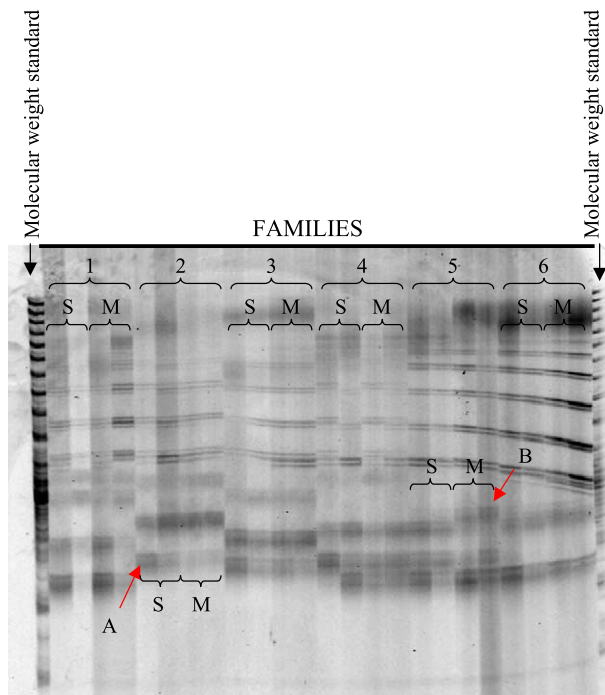


Fig. 3. Amplification products for microsatellite *Ssa94*—DNA pools for families 1 to 6. Each well contained pools from 10 individuals (M for mortalities and S for survivors). Each family was represented by two wells of 10 mortalities and two wells of 10 survivors (40 individuals in total). In family 2, one of the bands is noticeably more intense in the survivors than in the mortalities (indicated by arrow A). A difference in band intensity is also apparent in family 5 (arrow B).

lines, segregating markers were passed to the offspring by both the parents. The markers that were inherited from the dam were scored separately from those inherited from the sire, resulting in two genetic maps.

MapMaker®/Exp. 3.0 software package (Lander et al., 1987) was used to develop the linkage map. For the establishment of linkage groups, a Log of the Odds (LOD) score of 3.0 with a maximum distance of 50 centimorgans (cM) was used.

All loci were tested for two types of segregation distortion through a chi-square analysis (χ^2). First, it is expected that in the case of independent segregation of the marker and the QTL, bands will be distributed in a 1:1 ratio between the two groups: i.e., approximately 50% of the fish that died due to IHNV and the survivors would have the band. If a marker is associated with a QTL that affects resistance to IHNV, we would expect to observe a significant deviation from the 1:1 segregation ratio. The observed value for an allele is the sum of the number of individuals in which it is present in one of the groups and the number of individuals in which it is absent in the other. The expected value is the sum of the total individuals scored divided by two. Second, alleles were tested for Mendelian segregation distortion. The observed value for an allele is the sum of the number of the individuals in which it is present in the

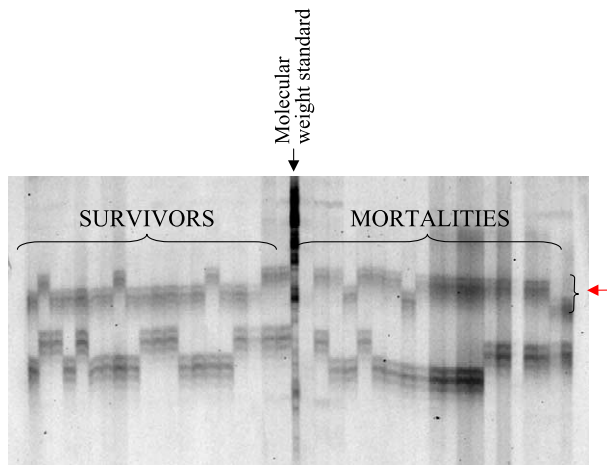


Fig. 4. Microsatellite *Ssa94* was amplified for the 40 individuals of family 5. The lower molecular weight alleles were transmitted by the dam and the higher molecular weight alleles (arrow) by the sire. There was a significant difference in frequency of the sire's alleles between the mortalities and survivor groups. The sire's lower

mortality and survivor groups. The expected value is the sum of all scored individuals divided by two. For both tests, deviations are considered to be statistically significant when $p < 0.05$.

3. Results

Challenge results are summarized in Table 2.

Table 2
Parental data and challenge results for individual families

Family	Dam ID	Sire ID	Progeny challenged (no.)	Mortalities (%)			Survivors (%)
				Days 1 to 10	Days 11 to 15	Days 16 to 21	
1	9	12	204	58.8	15.7	7.4	18.1
2	15	16	204	59.8	14.7	3.4	22.1
3	23	24	206	63.1	9.7	6.3	20.9
4	37	56	195	65.6	13.8	2.6	18.0
5	37	40	205	68.8	14.6	1.5	15.1
6	41	40	195	51.3	17.4	4.6	26.7
9	5	6	200	56.5	9.5	2.0	32.0
56	45	56	202	71.8	14.8	1.5	11.9
59	39	40	207	62.3	14.0	3.4	20.3
61	37	42	211	44.5	15.2	8.5	31.8

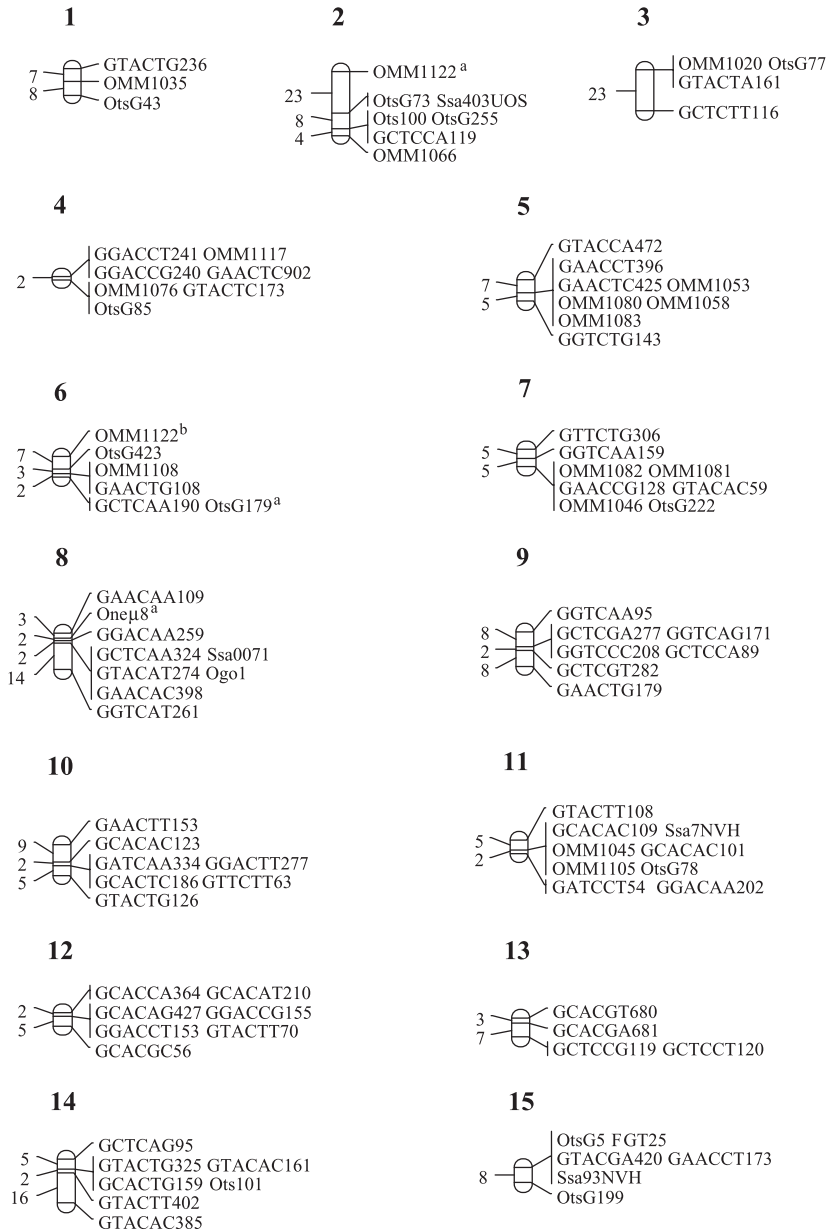


Fig. 5. Sire linkage map—all distance units are in centimorgans (cM). AFLP markers are represented by six letters followed by allele length; the remainders are microsatellites.

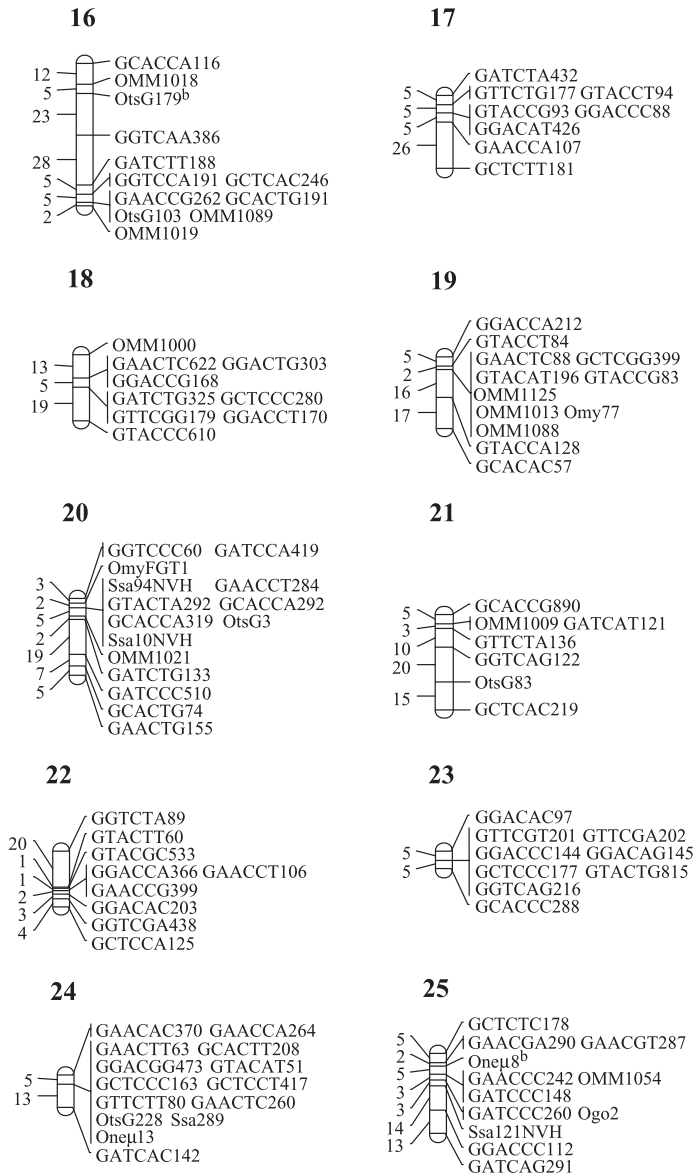


Fig. 5 (continued).

3.1. Family 9—linkage map

An average of 43.2 individuals was genotyped per marker, because some individual PCR reactions failed. Most microsatellites exhibited a Mendelian segregation ratio pattern. Markers presenting segregation distortion (μ Sat15, *OtsG423*, *OMM1084*, *OMM1108*, and

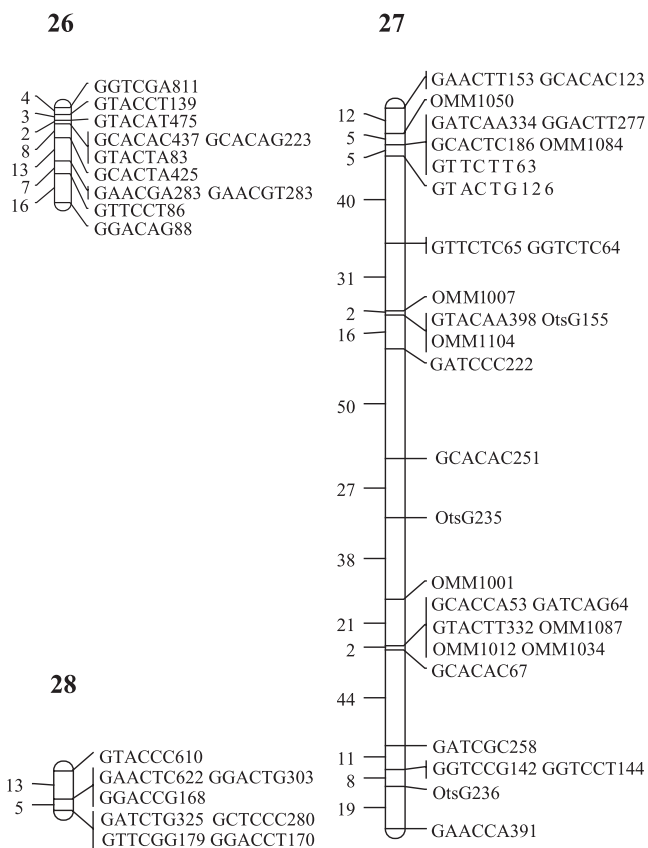


Fig. 5 (continued).

OMM1122) were double-checked and one unclear genotyped marker (μ Sat15) was eliminated. The sire map is composed of a total of 257 molecular markers, of which 185 are AFLPs and 72 are microsatellites. The markers are distributed over 28 linkage groups (Fig. 5), and cover 1019 cM, with an average distance of 3.9 cM. The dam map has a total of 236 markers, 164 AFLPs, and 72 microsatellite loci. It is composed of a total of 45 linkage groups (Fig. 6), covering 2041 cM, and averaging one marker every 8.6 cM. Forty-three microsatellites are common to both maps, 22 of which are linked in the sire and in the dam maps. Three microsatellites present more than one set of alleles in each map (sire—*Oneμ8*, *OMM1122*, and *OtsG179*; dam—*OtsG85*, *OMM1001*, and *OtsG179*). The minisatellite *Minisat* is also duplicated in the dam map.

3.2. Family 9: marker-QTL associations

In our first screening process with family 9, several markers showed substantial cosegregation with survivorship. The chi-square analysis indicated that 16 AFLPs

(GCACCA292, GGTCCC60, GAACCT 284, GCACTG74, GTACTA292, GATCCA419, GATCCC510, GAACGA290, GAACCC242, GAACGT 287, GGACCC112, GATCAG291, GATCCC260, GATCCC148, GCTCTC178, and GTACCA128) and seven microsatellites from the sire map (*FGT1*, *Ssa7NVH*, *OtsG78*, *Ogo2*, *Oneμ8^b*, *OMM1054*,

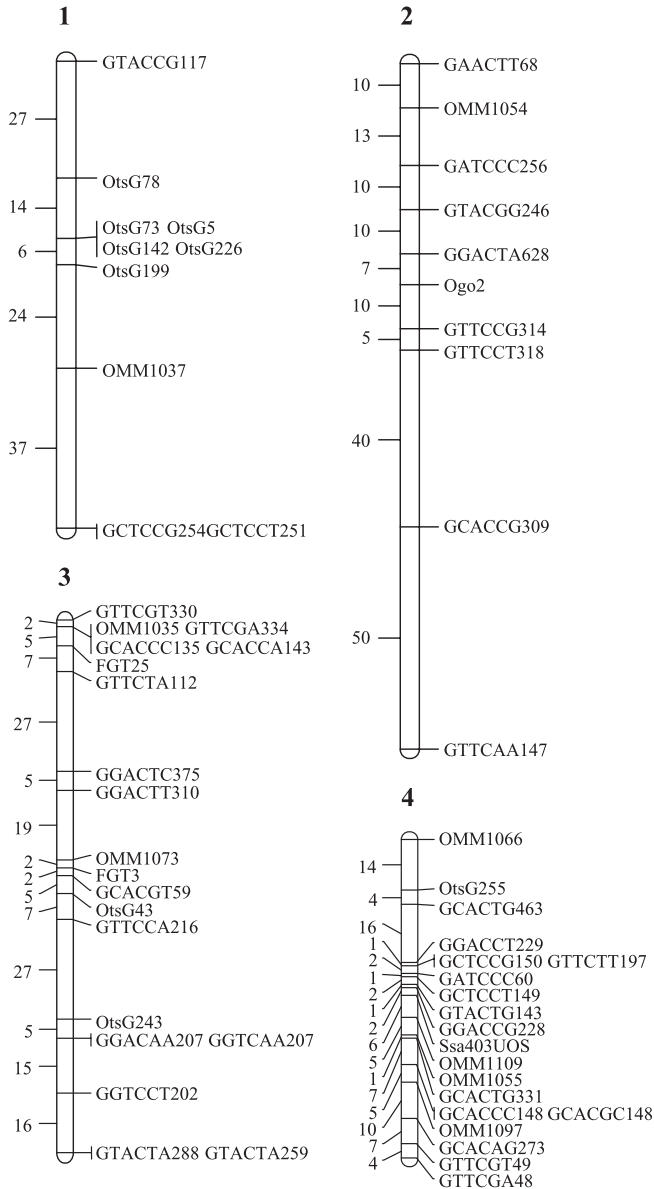


Fig. 6. Dam linkage map—all distance units are in centimorgans (cM). AFLP markers are represented by six letters followed by allele length; the remainders are microsatellites.

and *OtsG3*) were associated with IHNV resistance. Fifteen of the AFLPs and all seven microsatellites were located in linkage groups 11, 20, and 25 of the sire map (Table 3). Six AFLPs (GGTCAA207, GTTCTA486, GGACAC455, GAACCT124, GCACTC279, and

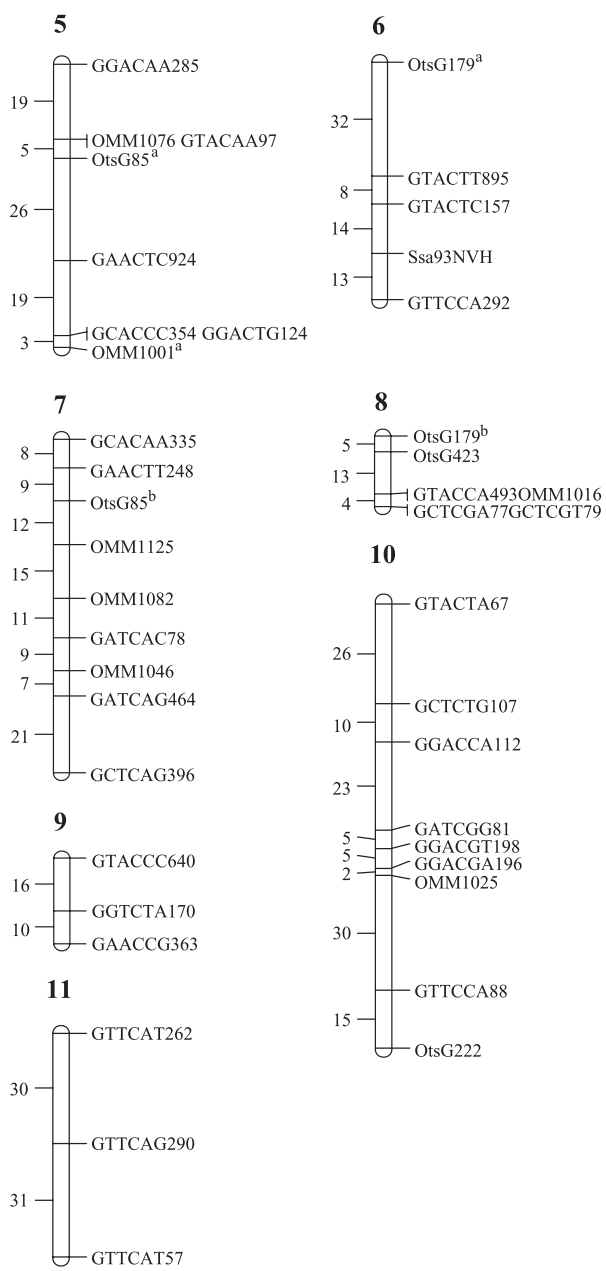


Fig. 6 (continued).

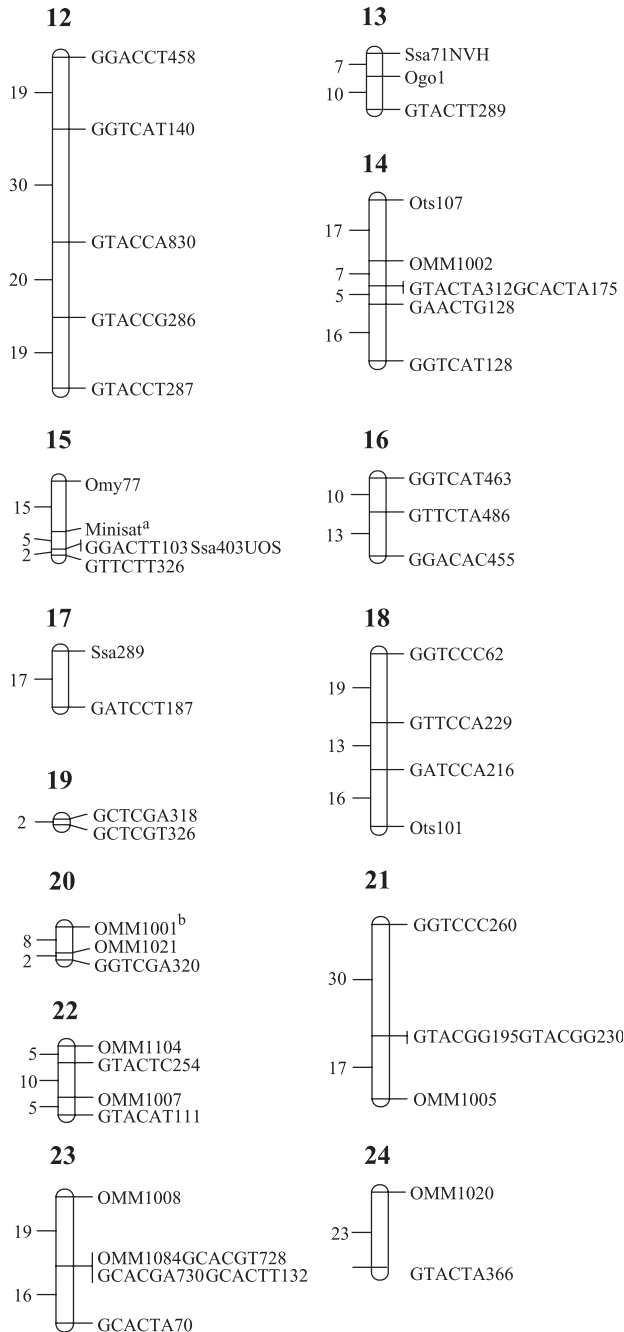


Fig. 6 (continued).

GCTCCA57) and six microsatellite markers in the dam map (*Ots*G243, *Ots*107, *OMM*1045, *OMM*1058, *OMM*1078, and *OMM*1083) showed association with IHNV resistance, and were located in five different linkage groups (3, 14, 16, 30, and 32).

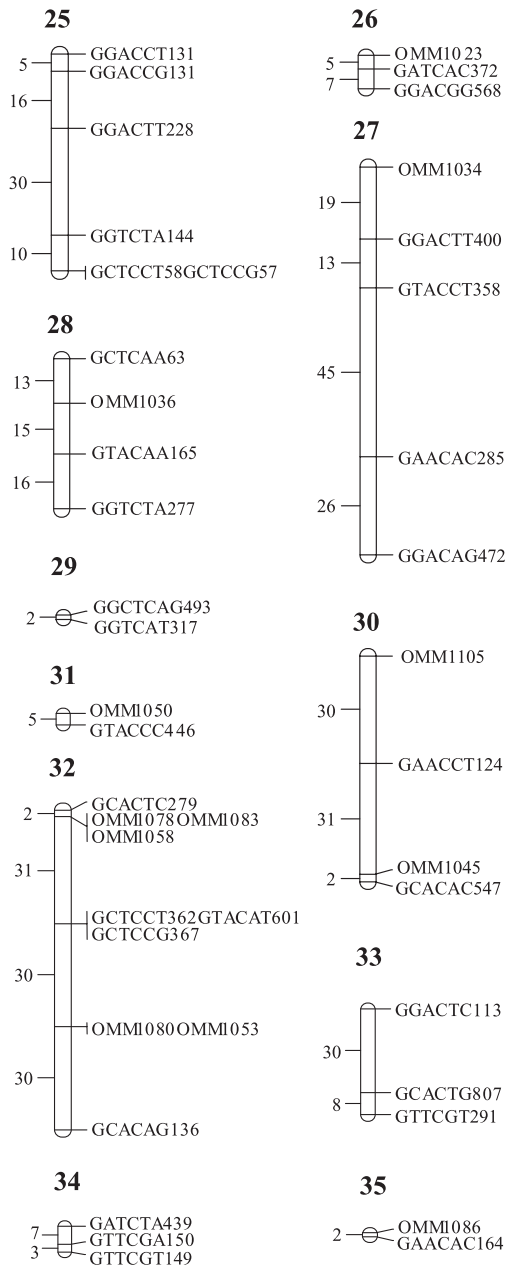


Fig. 6 (continued).

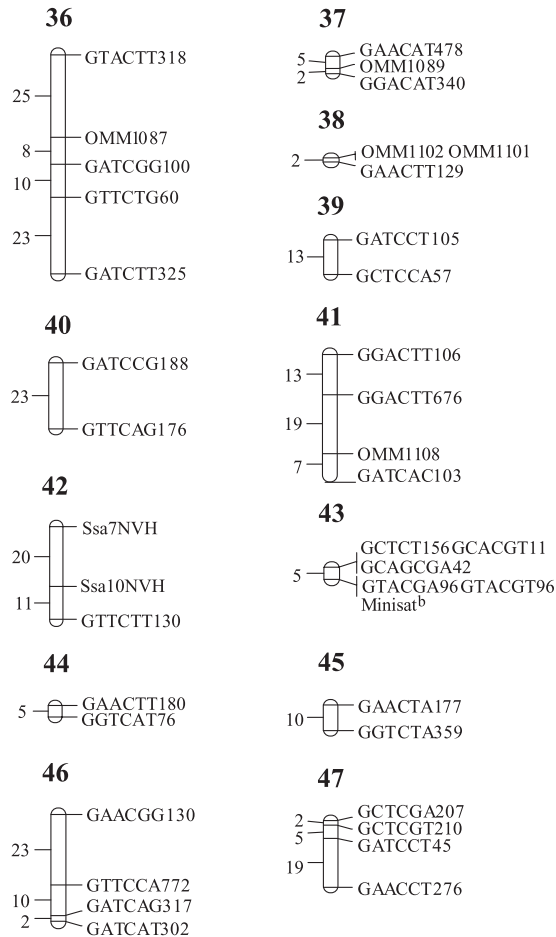


Fig. 6 (continued).

We augmented the sample size to 84 individuals (42 survivors and 42 mortalities) for *Ogo2*, *OMM1054*, *Ssa7NVH*, and *Oneμ8^b*, and observed an increase in the level of the statistical significance in three of them. *Ogo2* *p*-value increased in statistical significance, changing from 0.022 to 0.001, that for *OMM1054* decreased from 0.007 to 0.000, and that for *Ssa7NVH* from 0.047 to 0.002. The *p*-value for *Oneμ8^b* decreased in statistical significance, changing from 0.04 to 0.07. Table 3 shows *p*-values with larger sample size available.

3.3. Results replication

In order to verify the results obtained with family 9, we used all 13 microsatellites from the three sire linkage groups that had several markers showing association to

Table 3

Microsatellite–QTL association information in families 9 (sire), 2, 5, and 6

Family 9 (sire)				p-value (χ^2)					
Link. group	Markers	cM	p-value (χ^2)	Family2		Family5		Family6	
				♂	♀	♂	♀	♂	♀
11	<i>Ssa7NVH</i>	} Completely linked	0.002*						
	<i>OtsG78</i>		0.042*						
	<i>OMM1045</i>		0.070						
	<i>OMM1105</i>		0.070						
20	<i>FGT1</i>	} 2.5	0.010*			0.152	0.342		
	<i>Ssa94NVH</i>		0.064			0.001*	0.746		
	<i>OtsG3</i>	} 4.6	0.042*			0.435	0.876		
	<i>Ssa10NVH</i>		0.078						
	<i>OMM1021</i>	+	0.170						
25	<i>Oneu8^b</i>	+	0.070	0.114	0.206			1	0.027*
	<i>OMM1054</i>	+	0.000*	0.013*	0.068			0.023*	0.746
	<i>Ogo2</i>	+	0.001*	NI	NI			NI	0.006*
	<i>Ssa121NVH</i>	+	0.170						

*Loci associated with IHNV resistance by results of Chi-square test (χ^2 ; $p < 0.05$)

NI—band pattern was not informative, meaning marker was not polymorphic, or bands could not be assigned to either the male or the female because they shared the same allele.

IHNV resistance to analyze six other BC₁ families. The analysis also included the microsatellite loci that were not significantly associated with IHNV resistance in family 9 (Table 1). Of the 13 microsatellites used in the prescreening process, two were found to be linked in family 4 (*OMM1021* and *OtsG3*) and four (*Oneu8^b*, *OMM1054*, *Ogo2*, and *Ssa94*) were associated with regions of the genome affecting the trait of interest in at least one of the six families. Linkage group 25 best replicated the results: three of the microsatellites that are associated with IHNV resistance in family 9 also were associated with the trait in either family 2 or 6. *Omy1054* showed association in families 2 and 6, and *Oneu8^b* and *Ogo2* in family 6. *Ssa94* (linkage group 20) was associated with IHNV resistance in family 5. Both *Oneu8^b* and *Ogo2* alleles associated with resistance were inherited from the dam, whereas in *Ssa94* and *OMM1054* the allele for resistance was transmitted to the offspring by the sire (Table 3). Several of the microsatellites could not be evaluated in all families because the parents shared both alleles or were homozygous.

No association between IHNV resistance and the microsatellites that showed association in families 5 and 6 (*Ssa94*, *OMM1054*, *Ogo2*, and *Oneu8^b*) was detected in families 56, 59, or 61. Those families were related to families 4, 5, and 6 (Fig. 2).

A strong interaction with dam alleles was detected in the mode of resistance or susceptibility inherited from sire 40, which sired families 5, 59, and 6 with three different dams. One of the *OMM1054* alleles transmitted by the sire was associated to IHNV resistance in family 6, but not in families 5 and 59. Similarly, an *Ssa94* allele associated with IHNV resistance in family 5 was not in the other two families.

4. Discussion

IHNV is one of the major causes of economic losses to the rainbow trout industry; therefore, there is significant interest in developing lineages that exhibit improved resistance to this disease. The development of a genetic map is the first step towards molecular marker-based analyses for identification of QTLs associated with IHNV resistance, in turn providing the basis for future marker-based selection. This study identified molecular markers putatively associated with QTLs affecting IHNV resistance in different rainbow trout families, and added 61 new more microsatellite markers to the existing rainbow trout genetic maps.

There are currently three published rainbow trout linkage maps based on DNA markers (Young et al., 1998; Sakamoto et al., 2000; Nichols et al., 2003). The Nichols et al. (2003) map is an update of that of Young et al. (1998), and it is currently the most complete rainbow trout. Our maps had 5 microsatellites in common with Sakamoto et al. (2000) and 28 with Nichols et al. (2003), 2 of which were shared by all three studies (*FGT3* and *FGT25*). Eight of the linkage groups in Nichols et al. had at least two microsatellites that were informative in our families. Consistency was found in six of those linkage groups, as the markers were found linked in one of our maps (Table 4). The discrepancies observed between the maps may be partially explained by residual homology, providing four copies of some chromosomal regions. There is substantial evidence that salmonids have evolved from a tetraploid ancestor, resulting in genomes that have been separated into two different chromosome sets by genome diploidization (Wright et al., 1983; Allendorf and Danzmann, 1997). However, some of the distal regions of homeologous chromosome arms retain homology from crossing over during multivalent pairing events in males. Nichols et al. used duplicated microsatellite information to establish putative linkage group homeologies. For instance, *FGT3* and *FGT25* were linked in our dam map and in Nichols et al.; however, they were unlinked in Sakamoto et al. (2000). *FGT25* is duplicated in Nichols et al. and maps to linkage groups II and XXIX, suggesting homeology between them and potentially explaining some of the inconsistency between the studies. *FGT3* and *FGT25* mapped to linkage group XXIX in Nichols et al., and they may have been mapped to the correspondent homeologous linkage groups in Sakamoto et al. (2000; Table 4). Besides homeology, difference in genetic background (i.e., our map includes steelhead trout genetic material) and pseudolinkage (explained below) may explain some of the discrepancies between our maps and the previously published maps.

Despite the significant economic impact that IHNV exerts on the industry, there are few published genetic linkage studies on IHNV resistance in rainbow trout (Palti et al., 1999, 2001; Khoo, 2000). Khoo (2000) identified six AFLPs and four microsatellites associated with IHNV resistance. One of their microsatellites, *FGT25*, was mapped in family 9, but did not exhibit association with the trait in our study, possibly because the QTL identified by Khoo (2000) was not segregating in this family.

Eighteen AFLPs and six microsatellites mapped in family 9 showed association with IHNV resistance, and most of them were located in three linkage groups. The results obtained in families 2, 5, and 6 supported our findings for family 9. These results

Table 4

Microsatellites common among current maps and previously published genetic maps—linkage information

Microsatellite locus	Linkage groups			
	Nichols et al. (2003)	Sakamoto et al. (2000)	Family 9	
			SIRE	DAM
<i>FGT25</i>	OA-II	Oii	15	3
<i>OMM1035</i>		—	1	3
<i>OMM1037</i>		—	—	1
<i>Ogo2</i>	OA-III	A	25	2
<i>OMM1046</i>	OA-VI	—	7	—
<i>OMM1012</i>	OA-VII	—	27	—
<i>OMM1034</i>		—	27	—
<i>OMM1009</i>	OA-VIII	—	21	—
<i>OMM1001</i>	OA-IX	—	11	42
<i>Ots107</i>		—	20	42
<i>Ssa7NVH</i>	OA-X	—	27	5/20
<i>Ssa10NVH</i>		—	—	14
<i>OMM1018</i>	OA-XI	—	16	—
<i>OMM1066</i>	OA-XII	—	2	—
<i>Ots100</i>		—	2	—
<i>OMM1020</i>	OA-XIII	—	3	24
<i>OMM1036</i>	OA-XV	—	—	28
<i>Omy77</i>	OA-XVI	—	19	15
<i>OMM1013</i>		—	19	—
<i>OMM1101</i>		—	—	38
<i>OMM1117</i>	OA-XVII	—	4	—
<i>OMM1021</i>	OA-XVIII	—	20	20
<i>OMM1025</i>	OA-XIX	—	—	10
<i>OMM1050</i>	OA-XX	—	27	31
<i>OMM1023</i>	OA-XXII	—	—	26
<i>Ssa289</i>		—	24	17
<i>OMM1045</i>	OA-XXIV	—	11	30
<i>FGT3</i>	OA-XXIX	2	—	3
<i>FGT25</i>		Oii	15	3
<i>Ogo1</i>	—	A	8	13
<i>Ssa71NVH</i>	—	A	8	—

strengthen our confidence that linkage groups 11, 20, and 25 represent genomic regions that carry genes affecting resistance to IHNV.

Differences in *p*-values were observed in completely linked markers and can be explained by failure to PCR amplify all samples and differences in sample size. For instance, on linkage group 11, markers *OMM1045* and *OtsG78* were considered linked by the mapping program but had different *p*-values. Out of 44 individuals screened for *OtsG78*, three failed to amplify and 40 out of the 41 scored individuals had the same genotype as *OMM1045*. Such differences may also be observed in markers that have different sample size. Microsatellites *Ssa7NVH* and *OtsG78* had originally a similar *p*-value of 0.042; however, the analysis of an additional 40 individuals for marker *Ssa7NVH* changed the *p*-value to 0.002.

Although we observed several of the marker-resistance associations in different families, we were unable to do so within families of the same sire. This lack of association across families of the same sire could be due to epistasis, which causes the phenotype to be a result of the interaction between more than one gene. An alternative explanation is that the QTL combination (dam/sire) was not informative for the association study. For instance, if the dam also carries a QTL allele that confers resistance to IHNV, it is not possible to observe the cosegregation of the trait and the sire's microsatellite allele. It is important to keep in mind that QTL-marker associations may be missed or have their statistical significance reduced because the degree of association is determined by both the distance between the marker and the QTL and the effect of the gene on the trait in question. Individuals carrying a QTL allele that has a moderate effect on resistance to IHNV may still be susceptible to the disease given that it does not provide 100% resistance.

From the six microsatellites showing association with IHNV resistance in family 9, only microsatellites *OMM1021* and *Otsg3* are linked (family 4). Pseudolinkage in the sire from family 9 may have caused the markers to appear linked, when in reality, they are on different chromosomes. Pseudolinkage is a phenomenon observed in male salmonids, including rainbow trout (May et al., 1980; Wright et al., 1980; May et al., 1982; Allendorf and Danzmann, 1997), and it can be interpreted as a residual tetrasomic inheritance (Wright et al., 1983). Pseudolinkage can be caused by pairing of homeologous chromosomes in a multivalent structure, resulting in the cosegregation of genes that in reality are not positioned on the same chromosome (Wright et al., 1980; May et al., 1982). It can be observed within species and happens with higher frequency in genomes from different species or subspecies (Wright et al., 1983; Johnson et al., 1987). This phenomenon may also explain the recombination suppression observed in the sire map. The dam-to-sire linkage distance ratio observed in the 22 microsatellites linked in both sexes is 7.6:1, a much higher value than that observed by Sakamoto et al. (2000) of 3.25:1. Given that the maps in this study are composed by two different strains, these results may suggest that pseudolinkage is intensified by the increased genetic background difference.

Multiple QTL mapping studies complement one another given that IHNV resistance is determined by numerous genes and such studies may generate information on different regions of the genome containing such QTLs. Mapping microsatellites is more useful than mapping AFLPs, because microsatellite information can be readily applied to other families and to studies in other populations. The addition of microsatellite markers to the rainbow trout map increases its resolution and facilitates QTL fine mapping. Coupled with the physical mapping of the trout genome, it will enable future positional cloning of the loci and ultimately, identification of the genes that determine the traits of interest. This information will help direct breeding scheme programs through marker-assisted selection and introgression of economically important genes.

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